REMARKS

Claims 1-11, 13-14, 17-18, 21, 24, and 26-27 are currently under consideration.

1. The Objection to Claim 1 and the Claim Rejections Under 35 U.S.C. §112, First and Second Paragraph Should be Withdrawn

The objection to claim 1 and the rejection of Claims 1-11, 13, 14 18, 21, 24 26 and 27 under 35 U.S.C. §112, first and second paragraph, are rendered moot in view of amended claim 1. In particular, the phrase "wherein the composition of heterogeneous sample is unknown prior to conducting the method" has been deleted from claim 1. Applicant respectfully requests that the objection to claim 1 and the rejections under 35 U.S.C. §112, first and second paragraph, be withdrawn.

2. The Claims Are Not Obvious in View of Minden, Nelson or Barry

The rejection of claims 1-11,13-14,17-18, 21, and 24-27 is maintained under 35 U.S.C. §103(a) as being unpatentable over Minden et al. WO 02/086081 A2 ("Minden") and Nelson et al. U.S. Patent 6,887,713 ("Nelson").

According to the Examiner, Minden teaches methods of identifying a protein via assigning (i.e. separating) binding reagents to designated locations on an array, detecting the binding patterns, and comparing the binding pattern to a reference set (i.e. characterizing; please refer to the abstract, paragraphs [0005-0012], [0028-0032], [0035-0044], [0072-0074], [0077], [00117], Figures 1-11, and Table 1). In addition, Minden is said to further teach (i) that the molecular weight or mass of the binding reagents can be determined and that spectrometry can be utilized; (ii) that more than one protein can have the same epitope thus the common epitopes (i.e. more than one) would bind to the same defined location; (iii) that the total protein content of a cell or tissue can be utilized as the protein mixture; (iv) that the protein mixture can be fragmented with various chemical or enzymatic methods including trypsin; (v) that trypsin cleavage forms a peptide or epitope (i.e. motif) with C-terminal lysine or arginine residues; (vi) that the peptides or epitopes (i.e. motifs) can be at least three amino acids in length and can have at least two variable amino acids; (vii) that arrays can have different binding molecules at spatially addressable locations which bind to different binding reagents; (viii) that the protein mixture may comprise all (i.e. at least 10% of the peptides) of the proteins and that the epitopes

cover the binding mixture; (ix) that the array can have 2-100 different proteins; (x) that the binding reagents can be antibodies; (xi) that the proteins are compared to a reference set (i.e. characterizing; (xii) that the reference set can include prediction about binding based on the predicted digests of a protein mixture; (xiii) that various binding reagents can be compared to a reference set or to other binding reagents.

According to the Examiner, although Minden does not specifically teach determining the abundance of the proteins by the use of desorption mass spectrometry or collision induced dissociation mass spectrometry, for present claims 1, 24, and 26, Nelson teaches analyzing complex biological mixtures utilizing "lab-on-a-chip" (i.e. chip-based microarrays) and MALDI-TOF (i.e. combination of both desorption mass spectrometry and collision induced dissociation mass spectrometry) wherein the proteins are quantified (i.e. abundance), internal reference standards are utilized, and determining the amount (i.e. abundance) of the proteins. According to the Examiner, the claims would have been obvious because the substitution of one known element (i.e. mass spectrometry providing mass information only) for another (i.e. mass spectrometry providing both mass and abundance information; MALDI-TOF) would have yielded predictable results to one of ordinary skill in the art at the time of the invention and/or (b) the claim would have been obvious because a particular known technique (i.e. MALDI-TOF utilized to determine mass and abundance of proteins) was recognized as part of the ordinary capabilities of one skilled in the art. See *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007).

The rejection of claims 1-11, 13-14, 17-18, 21, 24, and 26-27 is maintained by the Examiner under 35 U.S.C.§ 103(a) as being unpatentable over Minden and Barry et al. WO 0225287 ("Barry"). Barry is said by the Examiner, to teach methods of determining the binding and mass of trypsin digested proteins including antibodies from a cell including phage or tissue sample immobilized on an array. According to the Examiner, it would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the method of identifying proteins taught by Minden with the MALDI-TOF analysis taught by Barry.

Applicant maintains that, for reasons detailed below, the present invention is not render obvious by Minden, Nelson or Barry, either alone or in combination. Specifically, combining the teachings of Minden and Nelson or Minden and Barry will not provide the same end-results as provided by the presently claimed invention, because the disclosed methods are fundamentally different. Minden discloses a method that is used to select and/or eliminate specific binders

(whether the method of Barry or Nelson is used). In contrast, the present invention is utilized to analyse the composition of complex protein-based heterogeneous constituents. It is noteworthy that the method of Minden (whether combined with the method of either Barry or Nelson) will simply not give the same end results as the present invention. The basis for this assertion is outlined in detail below and is depicted in attached Exhibits 1-3[1] which illustrate the results obtained when analysing a heterogeneous protein mixture (sample) composed of 10 proteinswhen the method of Minden (combined with either Nelson or Barry) versus the use of the present invention, .

In Minden's method (Exhibit 1), the sample of proteins is deposited in spots. The antibodies are then added one-by-one and if the binding motif is present, the antibody will bind specifically to the spot. Thus, information will only be obtained regarding whether the antibodies bound the spots or not as it is the bound antibody that is detected. As soon as ≥2 proteins/spot are deposited, i.e. in all cases when a mixture of proteins is targeted, one cannot determine to which of all the deposited proteins in each spot the antibody bound to, as binding motifs can be shared between different proteins. Hence, no information about the composition of the sample is generated.

In contrast to the method of Minden, in the instant invention, the antibodies are deposited one by one in unique spots (Exhibit 2). A sample of peptides/proteins is added to the array of immobilized antibodies. MS-MS is then used to determine which peptides/proteins are bound to each antibody, i.e. it is the bound peptides/proteins that are detected. Hence, crude mixtures can be addressed and information about the sample composition can be generated.

To summarize, Applicant maintains that the method of Minden and the method of the present invention comprise different steps that give different end results.

Furthermore, the methods are NOT interchangeable. In fact, for the method of Minden to be interchangeable with the method of the present invention, five significant limitations, i.e new circumstances, would have to be imposed on the method of Minden (whether combined with Nelson or Barry)---limitations which the instant invention would not be subjected to. Restated, these limitations must be considered if the inventions are to be assumed to be

[1] Applicants wish to note that Exhibits 1-3 were filed with the USPTO in color. When reviewing said Exhibits it is important that the Examiner review the Exhibits in color to more clearly understand the points depicted in the figures.

interchangeable. Applicant maintains that these five limitations, as discussed in detail below, help to clearly discriminate the claimed invention from the method disclosed by Minden.

The first limitation relates to sample complexity, i.e., a mixture of proteins/peptides vs. a single analyte. When the sample is composed of ≥ 2 proteins/peptides, i.e., every time a mixture is analysed, the method of Minden is unable to determine to which of the peptides/proteins in the array immobilized sample the antibody bound to, since the binding motif could be shared among different proteins. By introducing the limitation of only depositing one unique peptide/protein per spot this problem is potentially resolved, however, in practical terms, this means that Minden would have to pre-fractionate the sample, e.g., if composed of 100 proteins/peptides, into 100 fractions, each containing only one protein/peptide. The fractions would then be array immobilized and analysed separately. In contrast, the present invention is capable of generating information about the sample composition whether a mixture (≥ 2 proteins/peptides) or a single analyte is analysed. In practical terms, this means that one can analyse non-fractionated samples. To target a sample composed of e.g. 1-100 peptides/proteins, 1-100 antibodies (the precise number will depend on how the binding motifs are shared among different proteins), are array immobilized, the sample is added, and any bound peptides/proteins are detected and identified. Hence, the present invention is not subjected to a limitation regarding sample complexity.

The second limitation relates to sample identity, i.e., a known versus unknown composition. In the method of Minden, the sample is immobilized on the surface and then probed with antibodies (one by one). The bound antibody is then detected. This means that Minden is not be able to determine which protein/peptide the antibody bound to if i) the sample is composed of ≥ 2 proteins/peptides, and ii) if the sample is composed of a single (1) protein/peptide of unknown identity, since the binding motif can be shared among different proteins. In practical terms, this means that Minden would not only have to pre-fractionate samples composed of , e.g., 100 proteins/peptides, into 100 fractions, each containing only one protein/peptide, but Minden must also á priori determine the identity of each of these proteins/peptides. This could be accomplished by traditional well-known proteomic technologies, in which the sample is first pre-fractionated (e.g. LC or 2D-gels) and then analysed by MS-MS. However, doing this, would mean that the method of Minden is no longer needed as the sample has already been analysed and identified, i.e., the composition has already been determined. In contrast, for the present invention, the antibodies are array immobilised and then exposed to the sample. The bound peptides/proteins are then detected and identified. Thus, the

present invention generates information about the composition of the sample independent of whether (i) a mixture or a single analyte is analysed, and/or ii) whether the composition of the sample is unknown or known (i.e. á priori determined). Hence, the present invention, unlike the method of Minden, is not limited by the sample identity.

Yet another limitation relates to assay design, ie., the need for several steps vs. a single step. In order to accommodate the first and second limitation as set forth above, Minden would have to pre-fractionate the sample, e.g. composed of 100 proteins/peptides into 100 fractions, each containing one unique protein/peptide only. These fractions would then be individually array immobilized. Probing the array fractions with, e.g., 100 antibodies would then mean that each unique spot would have to be exposed to the antibodies one by one in individual assays. In other words, the assay would have to be run in 100x100 individual steps. Hence, the invention by Minden is subjected to a limitation requiring multiple steps. This is not a limitation of the present invention, as the invention is capable of analysing a sample mixture composed of, e.g., 100 proteins/peptides with, e.g., 100 antibodies in a one step approach. Specifically, the 100 antibodies would be array immobilized into unique spots. The entire array would then be simultaneously exposed to the sample, and any bound peptides/antibodies per spot would then be detected and identified.

A fourth limitation relates to sample complexity – 2-100 vs. >200 proteins/polypeptides. Although Minden states that their method could target samples composed of 2-100 different proteins/peptides, in practical terms, this means that the sample would have to be i) pre-fractionated into 2-100 fractions, each containing one unique protein/peptide, ii) the identity of each protein/peptide would have to be á priori determined, and iii) unique fractions would then be array immobilized and exposed to, e.g., 100 antibodies in 100x100 individual steps (assays). In contrast, the present invention is capable of targeting very heterogeneous mixtures composed of numerous proteins. In practical terms, this means that any number of required probes is array immobilized on the same array, and then simultaneously exposed to the same sample. To set a level significantly higher than Minden, one could define the invention as capable of targeting sample mixtures composed of >200 different proteins/peptides. Hence, the present invention is not subjected to a limitation in sample complexity.

Finally, a fifth limitation relates to the binding motif and whether it is shared or unique. In the Minden method, the sample is array immobilized and then probed with antibodies, and the bound antibody is detected. Whether the sample is constitutes a mixture (≥2 different

proteins/peptides) or a single analyte of unknown identity, the invention of Minden is not able to provide any information about the sample composition, since the binding motifs can be shared among different proteins. This problem can be circumvented by limiting the binding motifs to be non-shared, in other words to be truly unique and thus be present in only one (1) protein/peptide. In practical terms this, means that you would have to generate one antibody per peptide/protein. Thus, targeting the non-redundant human proteome composed of 20,300 proteins would require 20,300 specific antibodies. In contrast, in the present invention, the antibodies are immobilized, and the entire array is then exposed to the same sample, where after any bound proteins/peptide per antibody spot is determined and identified. In practical terms, this means that one can generate information about the sample composition targeting mixtures (≥2 different proteins/peptides) or single analytes whether the binding motifs are shared or not. Thus, targeting the non-redundant human proteome composed of 20,300 proteins would require anything from 1 to 20,300 specific antibodies depending on how the binding motifs are shared between different proteins. Hence, the present invention is not subjected to a limitation regarding the binding motif

Thus, the combined teachings of Minden and Nelson, and Minden and Barry, are conceptually different and not interchangeable with the claimed invention and would give strikingly different end results. Hence, the claims are non-obvious over the combination of Minden and Nelson and, therefore, the rejection under 35 U.S.C. §103 should be withdrawn.

CONCLUSION

In view of the foregoing amendments and remarks, it is believed that the subject claims are in condition for allowance, which action is earnestly solicited. If, in the opinion of the Examiner, a telephone conference would expedite prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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Exhibit 1

Reagents

Antibodies: Two specific antibodies, antibody A and B, each recognizing a unique binding motif (motifs a and b).

antibody A  antibody B 

Sample: A heterogeneous mixture of 10 proteins (colour coded).
5 of 10 proteins carry the same binding motif (motif a - red) recognized by antibody A.
2 of 10 proteins carry the same binding motif (motif b - purple) recognized by antibody B.
3 of 10 proteins does not carry any binding motifs recognized by the antibodies

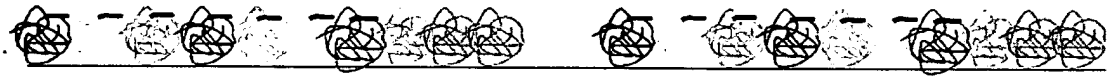
5 different proteins sharing binding motif a

2 different proteins sharing binding motif b

3 different proteins with no binding motif

Mindens approach

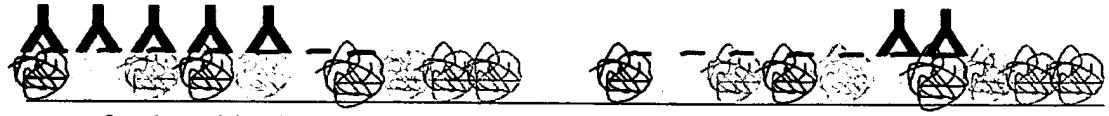
Step 1



Spot 1 containing the deposited sample

Spot 2 containing the deposited sample

Step 2



Spot 1 containing the deposited sample

Spot 2 containing the deposited sample

Step 3

Detection
Any antibody bound to the spot will be detected
- Antibody A bound spot 1

Detection
Any antibody bound to the spot will be detected
- Antibody B bound spot 2

Step 4-5

No information of which motif-containing peptides/protein that were that were present in the sample will be obtained. In other words, the data from spot 1 will state that antibody A bound to the spot, it will not state whether it bound the black, light blue, green, dark blue or yellow etc protein, or any combination thereof. Similar information will be obtained for spot 2.
Hence, the composition of the sample cannot be determined.

Exhibit 2

Current invention/approach

Step 1

Spot 1 containing Antibody A

Spot 2 containing Antibody B

Step 2

Spot 1 containing Antibody A

Spot 2 containing Antibody B

Step 3

Detection
Any bound peptides/proteins will be detected
-5 different proteins bound in spot 1
-The 5 bound proteins are identified

Detection
Any bound peptides/proteins will be detected
-2 different proteins bound in spot 2
-The 2 bound proteins are identified

Step 4-5

Direct information of which motif-containing peptides/protein that were present in the sample will be obtained. In other words, the data from spot 1 will tell which (identify) which peptides/proteins that were bound by antibody A (in any combination). Similar information will be ontained from spot 2.
Hence, the composition of the sample can be determined.

Exhibit 3

Mindens approach

1. The sample is (digested) and deposited in unqiue spots on the surface.

2. The antibodies are added in solution, one-by-one, and if the binding motif is present, the ab will specifically bind.

3. The bound antibodies are detected (using Nelson and/or Barry).

4. The output will simply be information on wether the ab had bound to the spot, and potentially which ab bound to which spot. It will NOT give any information to which motif-containing peptide(s) the ab bound to. Hence, no information will be generated regarding the composition of the sample.

5. The approach will only give information on whether the ab bound to the spot or not. As soon as 2 or more proteins per spot are deposited, the antibody binding patterns will not reveal from which protein the motif-containing peptide(s) originated, and thus not provide any information about the sample composition.

Current invention/approach

1. The antibodies are deposited in unique spots on the surface.

2. The sample is digested and added to the surface. Any motif-containing peptides will be specifically bound by the antibodies.

3. Using MS-MS, the motif-containing peptides will then be detected and identified (sequence). Based on this information, the wild type protein from which the motif containing peptides originated can be identified. Hence, detailed information about the composition of the sample will be generated.

4. Information of the sample composition can be generated for samples composed of several proteins, ranging from one to numerous (2,10,50, 100, 1000 etc).